The present study was approved by the Institutional Animal Care and Use Committee, Sun Yat-sen University, China (No. SYSU-IACUC-2020-000511), and Ethical Review Committee, Hospital of Stomatology, Sun Yat-Sen University, China (ERC-2013-15). This study followed the Ethical Principles of Animal Experimentation.

**Table S1. Antibodies**

|  |  |
| --- | --- |
| Antibodies | Purchased from |
| anti-PER2 primary antibody | Abclonal, Wuhan, China |
| anti-cytokeratin primary antibody | Boster Bio, Wuhan, China |
| anti-vimentin primary antibody | Boster Bio, Wuhan, China |
| Dylight 488/594 conjugated secondary antibody | EarthOx, CA, USA |
| anti-DSPP primary antibody | Santa Cruz, CA, USA |
| anti-DMP1 primary antibody | Affinity Biosciences, USA |
| anti-β-actin primary antibody | Beyotime, Shanghai, China |
| HRP-conjugated secondary antibodies (for western blotting) | Beyotime, Shanghai, China |

**Table S2. SiRNAs sequence information**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Species | Sense | Antisense |
| si-PER2-1 | Rat | 5’-GGAAAGGAGCUGCGGAUGUUATT-3’ | 5’-UAACAUCCGCAGCUCCUUUCCTT-3’ |
| si-PER2-2 | Rat | 5’-GGUGAAGGCUAAUGAGGAGUATT-3’ | 5’-UACUCCUCAUUAGCCUUCACCTT-3’ |
| si-PER2-3 | Rat | 5’-GCAAGUGAUCGAGGACUAAGATT-3’ | 5’-UCUUAGUCCUCGAUCACUUGCTT-3’ |

**Table S3. MIQE checklist**

|  |  |  |
| --- | --- | --- |
| *Item* *to* *Check* | *Importance* | *Checklist* |
| Experimental Design |
| Definition of experimental and control groups | E | Figure 4a: Experimental groups: pcDNA3.1-PER2. Control group: pcDNA3.1-NC.Figure 4b-d: Experimental groups: Control-oe-PER2; OS-oe-NC; OS-oe-PER2. Control group: Control-oe-NC.Figure 5a: Experimental groups: si-PER2-1; si-PER2-2; si-PER2-3. Control group: si-NC.Figure 4b-d: Experimental groups: Control-si-PER2; OS- si-NC; OS- si-PER2. Control group: Control- si-NC. |
| Number within each group  | E | n = 3 all groups |
| Assay carried out by core or investigator’s laboratory?  | D | Investigator’s laboratory  |
| Acknowledgement of authors contributions  | D | Yes, see Contributions  |
| Sample |
| Description  | E | total RNA of dental papilla cells |
| Volume/mass of sample processed  | D | - |
| Microdissection or macrodissection  | E | Macrodissection  |
| Processing procedure  | E | See methods: RT-qPCR. |
| If frozen, how and how quickly?  | E | Extracted RNA was immediately reverse transcribed into cDNA and stored at -80°C |
| If fixed, with what and how quickly?  | E | Not fixed  |
| Sample storage conditions and duration  | E | -80°C until RNA extraction  |
| Nucleic Acid Extraction |
| Procedure and/or instrumentation  | E | See methods: RT-qPCR. |
| Name of kit and details of any modifications  | E | See methods: RT-qPCR. |
| Source of additional reagents used  | D |  |
| Details of DNase or RNase treatment  | E | DNase from Yishan Biotechnology, Shanghai, China. Use RNAase-free consumables and reagents. |
| Contamination assessment (DNA or RNA)  | E | Use DNase during RNA extraction Electrophoresis assay |
| Nucleic acid quantification  | E | NanoDrop One Microvolume UV-Vis Spectrophotometers (Thermo Scientific) |

|  |  |  |
| --- | --- | --- |
| Yield  | D | - |
| Instrument and method  | E | NanoDrop One Microvolume UV-Vis Spectrophotometers (Thermo Scientific) |
| Purity(A260/A280)  | D | A260/A280 = 1.98-2.04 |
| RNA integrity: method/instrument  | E | Run an aliquot of the RNA sample on a denaturing agarose gel stained with ethidium bromide (EtBr) |
| RIN/RQI or Cq of 3’ and 5’ transcripts  | E | N/A |
| Electrophoresis traces  | D | - |
| Inhibition testing (Cq dilutions, spike, or other)  | E | Not performed, Input proper amount of RNA into the reverse transcription system, which minimises PCR inhibition  |
| **Reverse Transcription** |
| Complete Reaction Conditions  | E | Hifair® Ⅲ 1st Strand cDNA Synthesis SuperMix for qPCR (Yeasen, Shanghai, China);25℃ (5 min)-55℃ (15 min)-85℃ (5 min)-4°C (until removal). |
| Amount of RNA and reaction volume  | E | 1μg RNA; 20 μL reaction volume |
| Priming oligonucleotide (if using GSP) and concentration  | E | N/A |
| Reverse transcriptase and concentration  | E | 4×Hifair® Ⅲ SuperMix plus, concentration not stated (Yeasen, Shanghai, China); |
| Temperature and time  | E | 25℃ (5 min)-55℃ (15 min)-85℃ (5 min)-4°C (until removal). |
| Manufacturer of reagents and catalogue numbers  | D | - |
| Cqs with and without reverse transcription  | D | - |
| Storage conditions of cDNA  | D | Stored at -80°C |
| **qPCR Target Information** |
| Gene symbol  | E | *PER2, DSPP, DMP1, ALP*reference gene = *GADPH* |
| Sequence accession number  | E | *PER2* (NM\_031678.2)*DSPP* (NM\_012790.3)*DMP1* (NM\_203493.4)*ALP* (NM\_013059.2)*GADPH* (NM\_017008.4) |
| Location of amplicon  | D | - |
| Amplicon length  | E | *PER2* (129 bp)*DSPP* (109 bp)*DMP1* (499 bp)*ALP* (141 bp)*GADPH* (138 bp) |
| In silico specificity screen (BLAST, and so on)  | E | NCBI Primer BLAST |
| Pseudogenes, retropseudogenes, or other homologs?  | D | - |
| Sequence alignment  | D | - |
| Secondary structure analysis of amplicon  | D | - |
| Location of each primer by exon or intron (if applicable)  | E | N/A |
| What splice variants are targeted?  | E | The primers did not target specific splice variants, almost all splice variants were targeted. |
| **qPCR Oligonucleotides** |
| Primer sequences  | E | See Table 1. |
| RT Primer DB identification number  | D | -  |
| Probe sequences  | D | -  |
| Location and identity of any modifications  | E | N/A |
| Manufacturer of oligonucleotides  | D | - |
| Purification method  | D | - |
| Complete reaction conditions  | E | See below |
| Reaction volume and amount of cDNA/DNA  | E | Reaction volume: 20 µL, Amount of cDNA: 10 ng  |
| Primer, (probe), Mg2+, and dNTP concentrations  | E | Primer concentration: 0.2 µMMg2+, and dNTP mixture contained in Hieff® qPCR SYBR Green Master Mix (No Rox) (Yeasen), concentrations unknown |
| Polymerase identity and concentration  | E | Hieff® qPCR SYBR Green Master Mix(No Rox) (Yeasen) contains Hieff® DNA Polymerase (Yeasen), concentration unknown  |
| Buffer/kit identity and manufacturer  | E | Hieff® qPCR SYBR Green Master Mix(No Rox) (Yeasen)   |
| Exact chemical composition of buffer  | D | - |
| Additives (SYBR Green I, DMSO, and so forth)  | E | Hieff® qPCR SYBR Green Master Mix(No Rox) (Yeasen) contains SYBR Green I  |
| Manufacturer of plates/tubes and catalogue number  | D | 96-well qPCR plate (Abclonal)Catalogue Number: AI02003 |
| Complete thermocycling parameters  | E | 95°C for 5 minutes followed by 40 cycles of 95°C for 10 seconds, 55-60°C for 20 seconds, and 72°C for 20 seconds. After 40 cycles, the melting curve analysis was run with cycling conditions of 95°C for 1 minute, 55°C for 30 seconds and 95°C for 30 seconds  |
| Reaction setup (manual/robotic)  | D | Manual  |
| Manufacturer of qPCR instrument  | E | Roche LightCycler® 96 System  |
| **qPCR Validation** |
| Evidence of optimisation (from gradients)  | D | - |
| Specificity (gel, sequence, melt, or digest)  | E | Melting curve analysis had a single peak.No template controls were run for each gene to detect contamination and primer dimer formation.  |
| For SYBR Green I, Cq of the NTC  | E | Cq for all NTCs was >33  |
| Calibration curves with slope and y intercept  | E | *PER2:* y = -3.488\**x*+36.888*DSPP:* y = -3.308\**x*+38.368*DMP1:* y = -3.522\**x*+39.637*ALP:* y = -3.283\**x*+29.818*GADPH*: y = -3.304\**x*+26.979 |
| PCR efficiency calculated from slope  | E | *PER2:* 93.5%*DSPP:* 100.6%*DMP1:* 92.3%*ALP:* 101.7%*GADPH*: 100.8% |
| CIs for PCR efficiency or SE  | D | - |
| r2of calibration curve  | E | *PER2 R2:* 0.9978*DSPP R2:* 0.9993*DMP1 R2:* 0.9988*ALP R2:* 0.9959*GADPH R2*: 0.9994 |
| Linear dynamic range  | E | The standards were diluted in 5 successive concentration gradients according to the multiplicity ratio (10ng, 1ng, 100pg, 10pg, 1pg). |
| Cqvariation at LOD  | E | - |
| CIs throughout range  | D | - |
| Evidence for LOD  | E | - |
| If multiplex, efficiency and LOD of each assay  | E | N/A |
| **Data Analysis** |
| qPCR analysis program (source, version)  | E | LightCycler® 96 SW 1.1 (Roche)  |
| Method of Cq determination  | E | Threshold was determined automatically by the software, and the threshold was used to determine the Cq values of samples  |
| Outlier identification and disposition  | E | qPCR reactions that failed (no amplification) were excluded from analysis  |
| Results for NTCs  | E | Cq>33 for all NTCs  |
| Justification of number and choice of reference genes  | E | Reference genes: *GADPH*N=3Reference gene showing no significant difference in different DPCs groups. |
| Description of normalization method  | E | See methods: RT-qPCR.  |
| Number and concordance of biological replicates  | D | - |
| Number and stage (reverse transcription or qPCR) of technical replicates  | E | The qPCR reaction was performed with three independent samples (three biological replicates) and three sub-wells (three technical replicates) |
| Repeatability (intraassay variation)  | E | High repeatability |
| Reproducibility (interassay variation, CV)  | D | - |
| Power Analysis  | D | - |
| Statistical methods for results significance  | E | See methods: Statistical analysis |
| Software (source, version)  | E | See methods: Statistical analysis |
| Cq or raw data submission with RDML | D | - |