

Supplementary Materials for

Inhibition of TRPA1 could ameliorates periodontitis by reducing Periodontal ligament cells
oxidative stress and apoptosis via PERK/eIF2 α /ATF-4/CHOP signal pathway

Qian Liu^{1,2,3#}, Shujuan Guo^{1,2,3#}, Yanli Huang^{1,2,4}, Xiuqun Wei^{1,2,3}, Li Liu^{1,2,3}, Fangjun
Huo^{1,2}, Ping Huang^{1,3}, Yafei Wu^{1,3*}, Weidong Tian^{1,4*}

Correspondence to: Yafei Wu, Weidong Tian; Email: yfw1110@163.com, drtwd@sina.com;

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Supplementary information

Table. S1 The primers sequences used in Real-time quantitative polymerase chain reaction

Gene		Sequence
Cas3	Forward	5'-CCAAAGATCATACATGGAAGCG-3'
	Backward	5'-CTGAATGTTTCCCTGAGGTTTG -3'
Cas9	Forward	5'-GACCAGAGATTCGCAAACCAGAGG-3'
	Backward	5'-AAGAGCACCGACATCACCAAATCC-3'
Bax	Forward	5'-CGAACTGGACAGTAACATGGAG-3'
	Backward	5'-CAGTTTGCTGGCAAAGTAGAAA-3'
Bcl-2	Forward	5'-GACTTCGCCGAGATGTCCAG-3'
	Backward	5'- GAACTCAAAGAAGGCCACAATC-3'
GRP78	Forward	5'-CAGTTGTTACTGTACCAGCCTA-3'
	Backward	5'-CATTTAGGCCAGCAATAGTTCC-3'
cymC	Forward	5'-GCCAATAAGAACAAAGGCATCA-3'
	Backward	5'-TTAAGTCTGCCCTTTCTTCCTT-3'
TRPM8	Forward	5'-TTCATCGTCTTCGCTTACTTCT-3'
	Backward	5'- GGCTTTTGTGTTGATCTTGACA-3'
TRPV1	Forward	5'-CTCACAGACAACGAGTTCAAAG-3'
	Backward	5'-CTTGTAGTAGCTGTCCGTGTAG-3'
TRPV4	Forward	5'-CCTACATCATCCTCACCTTTGT-3'
	Backward	5'-CTTCCTCAGGAATACGGGGAAG-3'
TRPA1	Forward	5'-CAGTGACCACAATGGCTGGACAG-3'
	Backward	5'-GTGCAGTGTTCCCGTCTTCATCC-3'
CHOP	Forward	5'-GAGAATGAAAGGAAAGTGGCAC-3'
	Backward	5'-ATTCACCATTCGGTCAATCAGA-3'
PERK	Forward	5'-CCAGTTTTGTACTCCAATTGCA-3'
	Backward	5'-CAGATACAGCTGGCCTCTATAC-3'
Cas7	Forward	5'-GCTGGGCAAATGCATCATAATA-3'
	Backward	5'-TGGCACAAGAGCAGTCATTATA-3'

HSP70	Forward	5'-GATATTGTGCAGTTGCCTACAG-3'
	Backward	5'-GGCTTCTTAAGAACAACCTTTCGG-3'

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Figure legend

Fig. S1 Cell viability test with drug concentration

Periodontal ligament cells derived from healthy were used in these tests. CCK8 assay was used to detect cell viability under different concentration of HC030031, 4-PBA, GSK2656157, EGTA ($n = 3$). 10uM HC030031, 500uM 4-PBA, 10uM GSK2656157, 500uM EGTA were used in this study. Data analysis were performed by using one-way ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Error bars represent mean \pm SEM.

Fig. S2 Periodontitis mice model and TRPA1 expression in periodontium

a, Representative Micro-CT images, H&E staining and immunohistochemical staining (red error indicated highly expressed TRPA1 proteins) of mice maxillary in health control and periodontitis group. b, Quantitative statistical analysis of alveolar bone loss in Micro-CT ($n = 5$). ALB: alveolar bone loss. Data analysis were performed by using one-way ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Error bars represent mean \pm SEM.

Fig. S3 Quantitative analysis of alveolar bone loss and changes of CRP and body weight

a, Quantitative statistical analysis of alveolar bone loss in buccal or in palatal of Micro-CT in Con, Perio, HC1 and HC3 groups ($n = 4$). b, Body weight change of mice in Con, Perio, HC1 and HC3 groups ($n = 4$). c, serum CRP levels change of mice in Con, Perio, HC1 and HC3 groups ($n = 4$). Data analysis were performed by using one-way ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Error bars represent mean \pm SEM.

Fig. S4 H&E staining of mice organs

Representative H&E staining images of mice organs (heart, liver, spleen, lung, kidney) in Con, Perio, HC1 and HC3 groups.







