

Title page

Naringenin ameliorates swine pulpitis by modulating immune response

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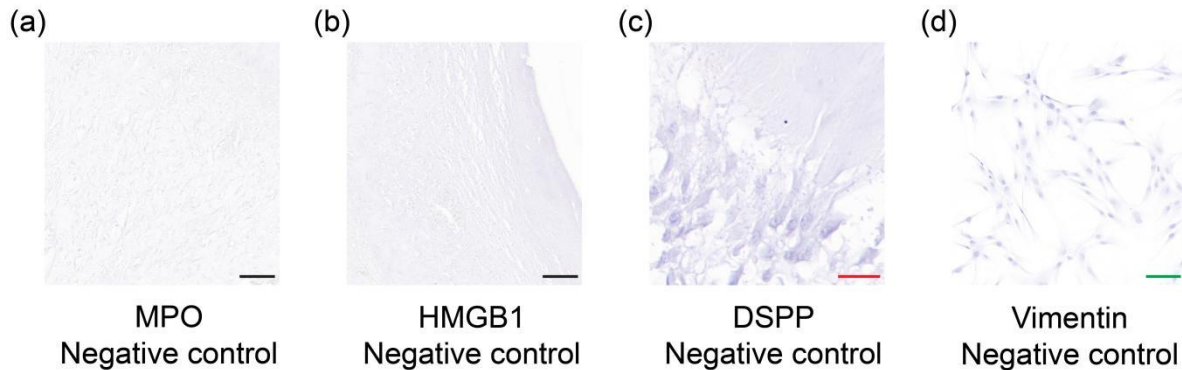
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Additional file 1

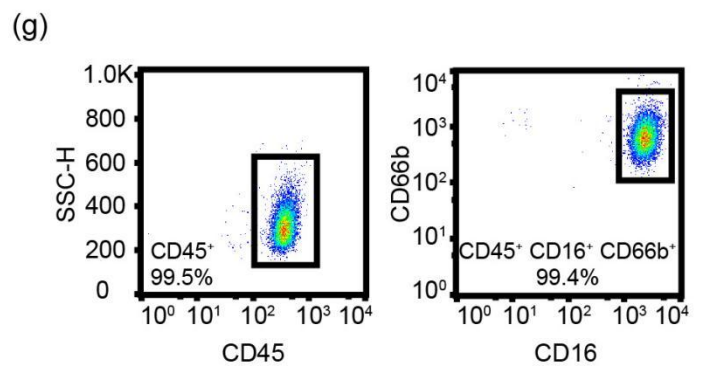
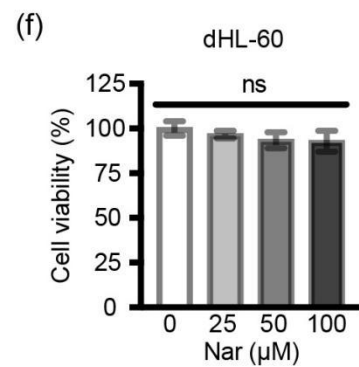
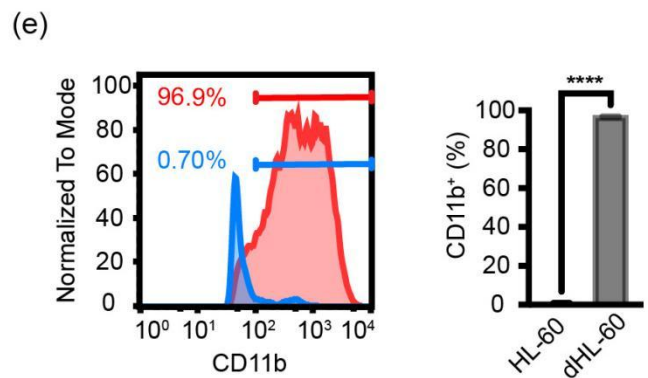
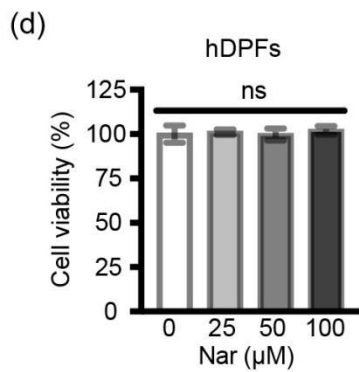
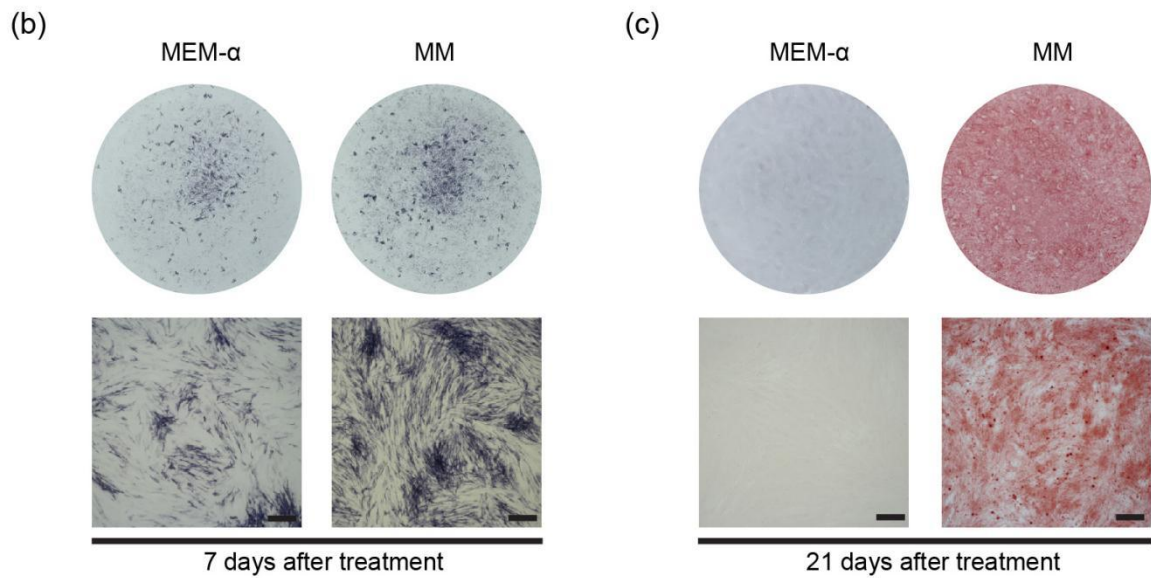
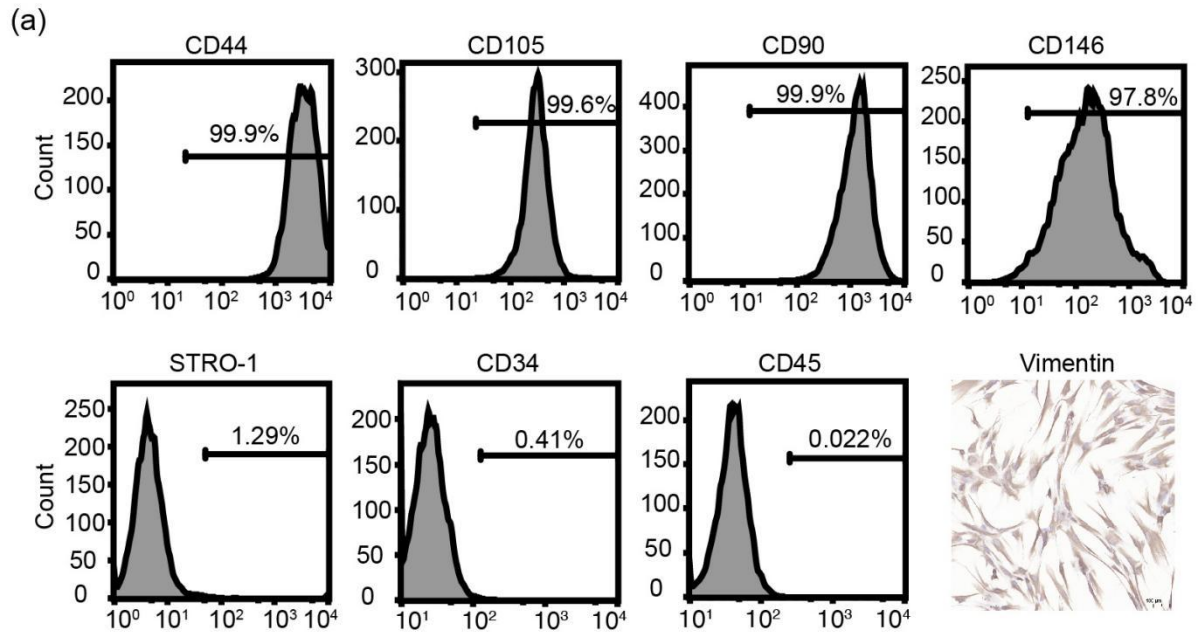
Antibodies

The primary antibodies were used in this study include: rabbit anti-MPO polyclonal antibody (22225-1-AP, Proteintech) at a dilution of 1:200; rabbit anti-HMGB1 polyclonal antibody (10829-1-AP, Proteintech) at 1:200; mouse anti-DSPP monoclonal antibody (sc-73632, Santa Cruz Biotechnology) at 1:200; and rabbit anti-vimentin polyclonal antibody (GB11192, Servicebio) at 1:500.

Secondary antibodies include: HRP-conjugated goat anti-mouse IgG (H+L) antibody (31430, Invitrogen) and HRP-conjugated goat anti-rabbit IgG (H+L) antibody (GB23303, Servicebio) were applied at a 1:200 dilution respectively.



Supplementary Fig. S1 Negative controls for immunohistochemistry and immunocytochemistry. Representative negative control images obtained by omitting the primary antibody for (a) MPO, (b) HMGB1, and (c) DSPP immunohistochemical staining in swine pulp tissue, and (d) vimentin immunocytochemical staining in dental pulp fibroblasts. No specific staining was observed in any control samples. Scale bars: 100 μm (green), 50 μm (black), 20 μm (red).



Supplementary Fig. S2 Cell preparation and identification of human dental pulp fibroblasts (hDPFs), differentiated HL-60 neutrophil-like cells (dHL-60), and human peripheral blood-derived neutrophils (hNeu.). **(a)** Purified hDPFs were identified through the FACS and ICC analyses. **(b)** Representative images of alkaline phosphatase (ALP) staining after 7-day treatment. **(c)** Representative images of Alizarin Red S (ARS) staining post 21-day treatment. Scale bars = 500 μ m. **(d)** CCK8 assays were used to assess the viability of hDPFs after treatment with 0-100 μ mol/L Nar for 24 hours. **(e)** dHL-60 cells were induced with DMSO for 7 days, and identified using the FACS analysis. **(f)** CCK8 assays were used to assess the viability of dHL-60 cells after treatment with 0-100 μ mol/L Nar for 24 hours. **(g)** Isolated hNeu. were identified through the FACS analysis. All results are shown as mean $\pm$ SD. **** $P < 0.0001$; ns indicates no significant difference.

Supplementary Table. S1 List of antibodies used for fluorescence-activated cell sorting (FACS) analysis.

Antibody	Clone number	Catalog number	Conjugate	Source
CD44 mAb	IM7	12-0441-81	PE	eBioscience
CD105 (Endoglin) mAb	SN6	12-1057-41	PE	eBioscience
CD90 (Thy-1) mAb	eBio5E10 (5E10)	17-0909-41	APC	eBioscience
CD146 mAb	P1H12	12-1469-41	PE	eBioscience
STRO-1 mAb	STRO-1	PE-65184	PE	ProteinTech
CD34 mAb	QBEnd-10	FITC-65183	FITC	ProteinTech
CD45 mAb	HI30	11-0459-41	FITC	eBioscience
CD16 mAb	eBioCB16 (CB16)	12-0168-41	PE	eBioscience
CD66b mAb	G10F5	17-0666-42	APC	eBioscience
CD11b mAb	ICRF44	17-0118-41	APC	eBioscience