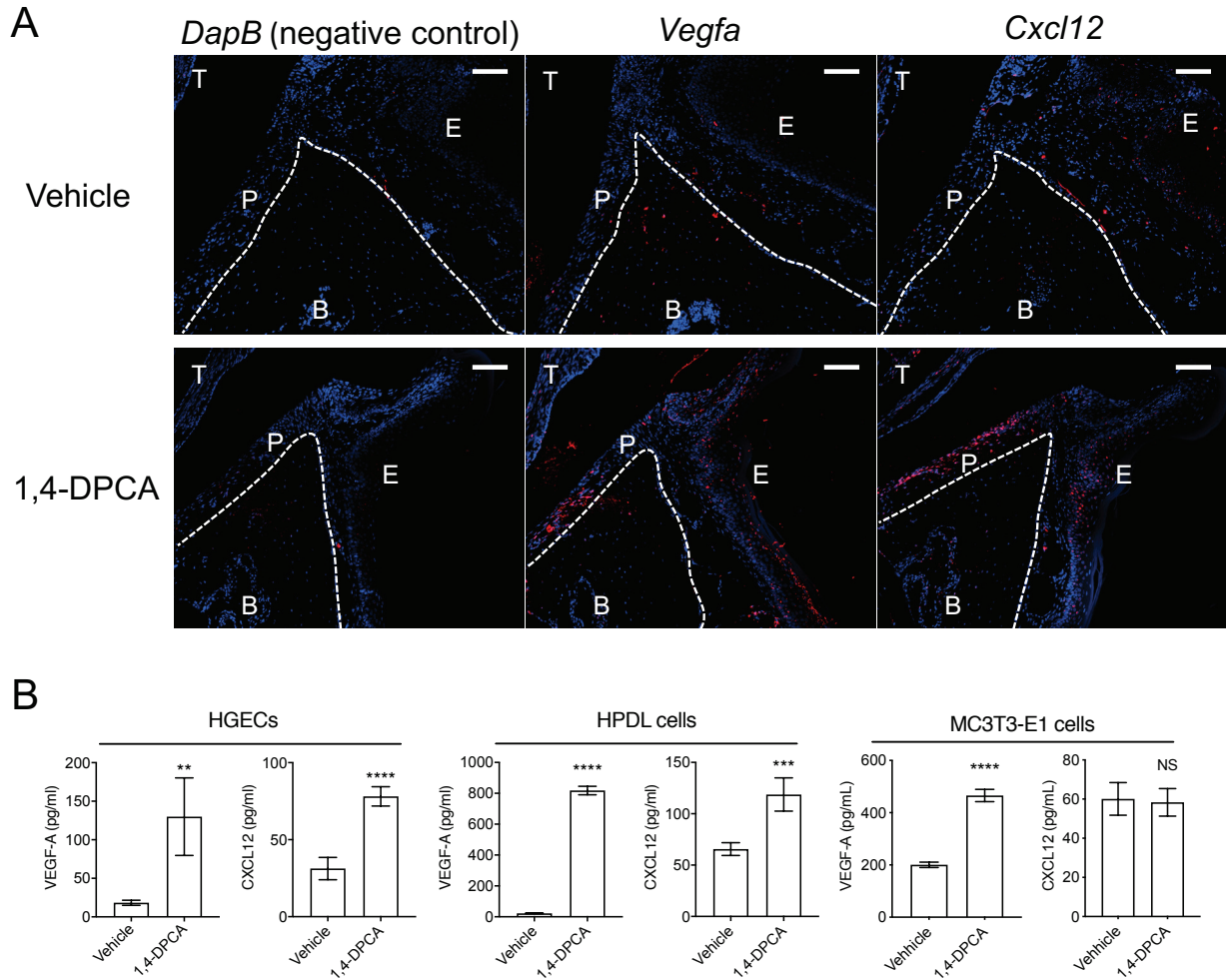


SUPPLEMENTAL FIGURES AND TABLE

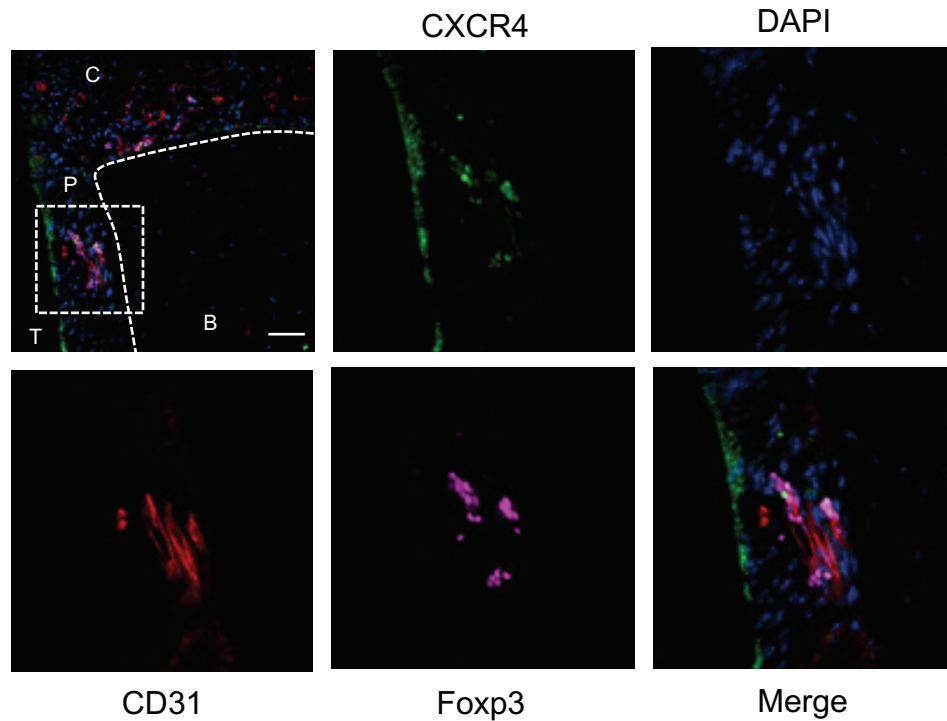
Nagai et al; An injectable hydrogel-formulated inhibitor of prolyl-4-hydroxylase promotes T regulatory cell recruitment and enhances alveolar bone regeneration during resolution of experimental periodontitis

Supplemental Figures

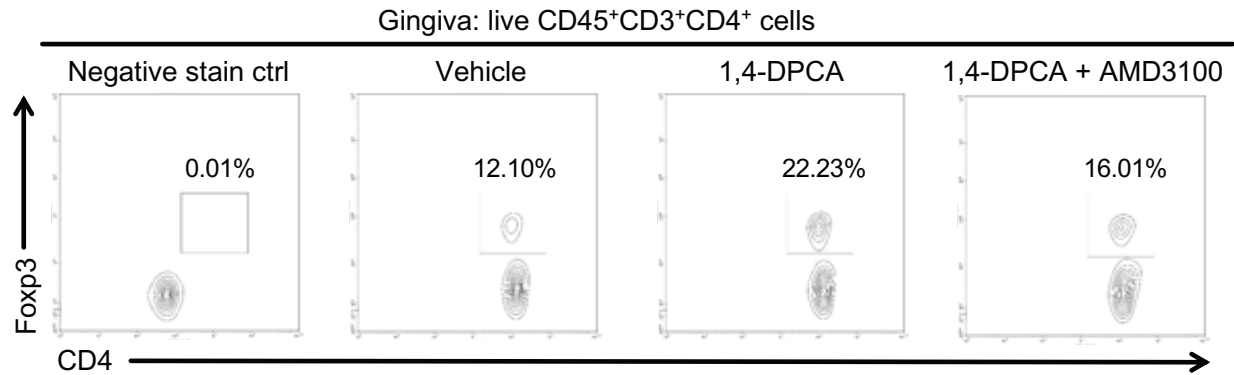


Supplemental figure S1: 1,4-DPCA/hydrogel induces VEGF and CXCL12 production in gingival epithelium and periodontal ligament during the resolution of periodontitis.

Mice were subjected to LIP for 10 days followed by 5 days without ligatures to enable resolution. At the onset of resolution (day 10), the mice were subcutaneously injected once with 1,4-DPCA/hydrogel or vehicle alone and were euthanized on day 15 for fluorescent histochemistry. Periodontal tissue sections were stained for mRNA expression of *Vegfa* (red), *Cxcl12* (red), and DAPI (blue) using RNAscope *in situ* hybridization. B, Bone; E, Epithelium; P, Periodontal ligament; T, Tooth. Scale bars, 100 μ m. (B) Human gingival epithelial cells (HGECs), human periodontal ligament (HPDL) cells, and MC3T3-E1 osteoblastic cells were stimulated with 1,4-DPCA (50 μ g/ml final concentration) or vehicle control (DMSO; 0.17% final concentration). After 24h, culture supernatants were collected and the levels of VEGF and CXCL12 were determined by ELISA. Data are shown as means \pm SD ($n=4$ cell cultures). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (Student's t test).



Supplemental figure S2: Colocalization of FoXP3⁺ Treg cells with CXCR4 and CD31 in the periodontal tissue of mice treated with 1,4-DPCA/hydrogel. Mice were subjected to LIP for 10 days followed by 5 days without ligatures to enable resolution. At the onset of resolution (day 10), the mice were subcutaneously injected once with 1,4-DPCA/hydrogel or vehicle alone and were euthanized at day 15 for fluorescent histochemistry. Shown is a periodontal tissue section stained for CXCR4 (green), CD31 (red), FoXP3 (purple), and DAPI (blue). B, Bone; C, Connective tissue; P, Periodontal ligament; T, Tooth. Scale bar in the top left image is 100 μ m; the rest are enlarged images of the square inset.



Supplemental figure S3: AMD3100 inhibits Treg cell accumulation in gingival tissue induced by 1,4-DPCA/hydrogel during the resolution of periodontitis. Groups of mice were subjected to LIP for 10 days followed by 5 days without ligatures to enable resolution. At the onset of resolution (day 10), groups of mice were subcutaneously injected once with vehicle alone (one group) or 1,4-DPCA/hydrogel (two groups). The 1,4-DPCA/hydrogel-treated groups were additionally intraperitoneally injected with AMD3100 or PBS control daily for 5 days. Gingival tissues were harvested on day 15 and processed for flow cytometric analysis to identify Foxp3⁺ Treg cells. Shown are representative FACS plots of Treg cells in the gingival tissue on day 15.

Supplemental Table S1: List of TaqMan gene expression primers (Thermo-Fisher Scientific).

Assay ID	Gene symbol
Mm00446190_m1	<i>Il6</i>
Mm00439618_m1	<i>Il17a</i>
Mm00443258_m1	<i>Tnf</i>
Mm00501584_m1	<i>Runx2</i>
Mm00475834_m1	<i>Alpl</i>
Mm03413826_mH	<i>Bglap</i>
Mm00475162_m1	<i>Foxp3</i>
Mm01178820_m1	<i>Tgfβ1</i>
Mm01288386_m1	<i>Il10</i>
Mm00437306_m1	<i>Vegfa</i>
Mm00445552_m1	<i>Cxcl12</i>
Mm01996749_s1	<i>Cxcr4</i>
Mm01545399_m1	<i>Hprt</i>