Supplemental data

Inclusive, Exclusive and Hierarchical Atlas of NFATc1⁺/PDGFR- α^+ Cells in

Dental and Periodontal Mesenchyme

Xue Yang^{1, 2, *}, Chuyi Han^{1, *}, Changhao Yu^{1, 2}, Bin Zhou³, Ling Ye^{1, 2, #}, Feifei Li^{1, 4, #},

Fanyuan Yu^{1, 2, #}

1 State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, China

2 Department of Endodontics, West China Hospital of Stomatology, Sichuan University, China

3 Center for Excellence in Molecular Cell Science, University of Chinese Academy of Sciences, Chinese Academy of Sciences, China

4 Department of Pediatric Dentistry, West China Hospital of Stomatology, Sichuan University, China

* These authors contribute equally.

Corresponding Author. E-mail: yeling@scu.edu.cn; feidepingfanshijie@163.com; fanyuan_yu@outlook.com



Figure 2—figure supplement 1. A. The tissue-clearing (TC) and whole-mount imaging procedure of mice maxilla. B. The images before & after the TC procedure of mice maxilla. C. The distribution of PDGFR-a+ and NFATc1+ cells from the section of XZ axis after 3D reconstruction of TC imaging. The image was from $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse). Box 1: alveolar bone. Scale bar = 300 µm for top row, 100 µm for bottom row.



Figure 3—figure supplement 1. The total 121 slices of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (pulse). The images were acquired by confocal microscope, ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.

Pdgfra/Nfatc1/DAPI Coronal pulp



Figure 3—figure supplement 2. Representative images of coronal pulp, root pulp, and PDL of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (pulse). The images were acquired by confocal microscope.



Figure 3—figure supplement 3. The tdTomato signal in pulp reconstructed by traditional serial section-based confocal imaging method (scale bar = 300 μ m). The sample was from $Pdgfra^{CreER} \times Nfatc 1^{DreER} \times LGRT$ mice (pulse).



Figure 3—figure supplement 4. The discontinuities in the z-axis due to stratification of slices. The image was reconstructed by serial sections of maxilla M1 of $Pdgfra^{CreER} \times Nfatc I^{DreER} \times LGRT$ mice sample (pulse).



Scale bar = 200 µm

Figure 3—figure supplement 5. 3D reconstruction of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse) by DICOM-3D; in PDL: ZsGreen⁺ cells in green, tdTomato⁺ cells in rose red; in pulp: ZsGreen⁺ cells in purple, tdTomato⁺ cells in blue. The image stack was also displayed in buccal view, coronal view, and radicular view of pulp and PDL, respectively.

			×		×.			350	
	Contraction of the second					1000			No.
Alex			ð						
			AUX)	Ø.	105		ZQ.		
		Net.							
		No.						ST REAL	1 Martin
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2 B							ý.

Figure 4—figure supplement 1. The total 88 slices of mandible M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (pulse). The images were acquired by confocal microscope, ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.



Figure 4—figure supplement 2. Representative images of coronal pulp (A) and PDL (B) acquired by confocal microscope of mandible M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (pulse). (Scale bar = 100 µm).



Figure 5—figure supplement 1. The number of PDGFR- α^+ cells, NFATc1⁺ cells, and PDGFR- α^+ &NFATc1⁺ cells in pulp and PDL, respectively. The quantification was done on a z stack of images in Imaris using the automatic spot detection feature.

	2										1 mont
					<u>Vici</u>		in.		j.	No.	No.
1 and					<u>is</u>						1 M
1	1000	I.S.					<u>IN</u>	<u>U</u>	in the	<i>Will</i>	
Carlo			Ser Stand		1 Miles	1 M	L BAR	L BA	L.	t fold	C All
A fait	No.	N. C.	No.		Not	See 1	Next.	Vet		Note:	Not
No.	Vet		₹1¢		N.	Nø.	Vø.	<u>No</u>	V		
							ila.	N F	and the second s	N.	N.
N.		O r	100 M	<u>کې</u>	۶Ÿ	\$	ð			La contraction de la contracti	
						And the second second	A CONTRACT	And a			

Figure 6—figure supplement 1. The total 117 slices of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (tracing). The images were acquired by confocal microscope, ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.

ZsGreen/tdTomato/DAPI



Figure 6—figure supplement 2. Representative images of coronal pulp, root pulp, and PDL of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (tracing). The images were acquired by confocal microscope (scale bar = 50 µm).



Figure 6—figure supplement 3. The tdTomato signal in pulp of maxilla M1 of $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (tracing) reconstructed by traditional serial section-based confocal imaging method (scale bar = 300 µm).



Figure 6—figure supplement 4. The discontinuities in the z-axis due to stratification of slices. The image was reconstructed by serial sections of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (tracing) using Imaris. ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.



Figure 6—figure supplement 5. 3D reconstruction of maxilla M1 of $Pdgfra^{CreER}$ ×

Nfatc1^{*DreER*} × LGRT mice (tracing) by DICOM-3D; PDL: ZsGreen⁺ cells in green, tdTomato⁺ cells in rose red; pulp: ZsGreen⁺ cells in purple, tdTomato⁺ cells in blue. The image stack was also displayed in buccal view, coronal view, and radicular view of pulp and PDL, respectively.

	and the second s										
									Ö.		C. And States
										101	
URS		lat	LQS.			ligg	lias	1000	1901	Mar.	M
Mail	ter	1/BU									
				W.					Store Barrier	and the	
	e e e	0 41 1	44	<u>.</u>	0.8	0.5	0.8		6.6		

Figure 7—figure supplement 1. All consecutive slices (a total of 120 slices) for imaging of mandible M1 of $Pdgfra^{CreER} \times Nfatc 1^{DreER} \times LGRT$ mice (tracing 11 days). The images were acquired by confocal microscope, ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.

<figure>

Figure 7—figure supplement 2. Representative images of coronal pulp (A) and PDL (B) acquired by confocal microscope of mandible M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice sample (tracing). Scale bar = 100 µm.

1 August	1	Star Star	A CONTRACTOR				19 ²	No.			and the second s
			AN AN		N.	S.	N.	35	T	N.	N.
X	N.	No.		ALC:				X	No.	STA .	
T.				- Marine	and the	No.	- Mi		and a	1 and	and the second second
and the	1 AM	and h	Control of	1 the	Contraction of the	1725	U.S.	Card to	erth	1/2	14
1/2	2005	C	1135	X	1	No.		TC.		and the second s	
eligs	1/e	1	91/28 S	110				rist.	N AS		
1/10	20	2.D	250	200	100						
			100		1.		1 de la companya de l		-250	(a)	<i>(</i> 58)
		120	1	1. Alexandre				(A)		1 Cal	1 cm
1990 - A.	1	100						- Che	- Alex		

Figure 8—figure supplement 1. All consecutive slices (a total of 130 slices) for imaging of maxilla of $Pdgfra^{CreER} \times IR1$ mice. The images were acquired by confocal microscope, ZsGreen⁺ cells in green and DAPI in blue.



Figure 8—figure supplement 2. All consecutive slices (a total of 121 slices) for imaging of maxilla of $Nfatc1^{DreER} \times IR1$ mice. The images were acquired by confocal microscope, tdTomato⁺ cells in red and DAPI in blue.

R.					Ţ	The second	The second se
100		T.	and the	Wie .	W	S.	
SCI RE							
			A CONTRACTOR				a de la compañía de l
			See See			A. C.	
Alton	20	SR	2 M				
	a sid			<u>Zaik</u>		a se	a file
(sel	1 gent				a contraction		
	C						
			190				12
				A Conta (Conta (Conta da	Jac I		12-51
			6-1-7		8.44 j	A Artemy	9/6/24
Lag	Andrew J.	6.89	Alban	2019	1	Strange	8.649 g
2° 19	Si Mij) · 64	Singl	5 <u>446</u>	1	5. * *	
de la	5449°					S.	
S				5	8.7%	Sanda	

Figure 8—figure supplement 3. All consecutive slices (a total of 127 slices) for imaging of maxilla of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times IR1$ mice sample. The images were acquired by confocal microscope, ZsGreen⁺ cells in green, tdTomato⁺ cells in red, DAPI in blue.



Figure 8—figure supplement 4. Representative images of coronal pulp (A) and PDL (B) acquired by confocal microscope of mandible M1 of $Pdgfra^{CreER} \times IR1$ (a1, b1), $Nfatc1^{DreER} \times IR1$ (a2, b2) and $Pdgfra^{CreER} \times Nfatc1^{DreER} \times IR1$ (a3, b3) mice. Scale bar = 100 µm.



Figure 8—figure supplement 5. 3D reconstruction of maxilla M1 by DICOM-3D of maxilla M1 of $Nfatc1^{DreER} \times IR1$ and $Pdgfr-a^{CreER} \times Nfatc1^{DreER} \times IR1$ mice; in PDL, ZsGreen⁺ cells in green, tdTomato⁺ cells in rose red; in pulp, ZsGreen⁺ cells in purple, tdTomato⁺ cells in blue. The image stack was displayed in buccal view, coronal view, and radicular view. Scale bar: 200 µm.



Figure 9—figure supplement 1. The identification of the types of PDGFR- α^+ & NFATc1⁺ cells (red) of dental pulp and periodontal ligament tissues. (A) Schematic illustration of lineaging tracing in $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LRTD$ mice. The mice were administrated with tamoxifen at D1 and D3, and sacrificed at D14. (B, C) Representative IF images of PDL B) and pulp C) of mandible M1 in P $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LRTD$ mice showing the MSCs marker AlphaV (left) and hematopoietic marker CD45 (right). Arrows B, C) indicate the co-localization of AlphaV/CD45 and tdTomato. D: dentin; AB: alveolar bone. P: PDL B), pulp C). Scale bar: 100 µm.

Legends of supplemental videos

Video 1. Panoptic multicolor imaging of PDGFR- α^+ cells(green) & NFATc1⁺ cells(red) in the pulp and PDL area of maxilla M1 of *Pdgfra*^{CreER} × *Nfatc1*^{DreER} × LGRT mice (pulse), the whole-tissue imaging was achieved through TC procedure, related to Figure 2C.

Video 2. Panoptic multicolor imaging of PDGFR- α^+ cells & NFATc1⁺ cells in the pulp and PDL area of maxilla M1 of $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse), the whole-tissue imaging was reconstructed from serial sections, related to Figure 3C. PDGFR- α^+ cells in green, NFATc1⁺ cells in red and DAPI in blue **Video 3.** Panoptic multicolor imaging of PDGFR- α^+ cells & NFATc1⁺ cells in the pulp and PDL area of mandible M1 of $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse), the whole-tissue imaging was reconstructed from serial sections, related to Figure 4C. PDGFR- α^+ cells in green, NFATc1⁺ cells in red and DAPI in blue

Video 4. Panoptic multicolor imaging of ZsGreen⁺ cells (green) & tdTomato⁺ cells (red) in the pulp and PDL area of maxilla M1 of $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (tracing 11 days), the whole-tissue imaging was achieved through TC procedure, related to Figure 5B.

Video 5. Panoptic multicolor imaging of ZsGreen⁺ cells & tdTomato⁺ cells in the pulp and PDL area of maxilla M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (tracing 11 days), the whole-tissue imaging was reconstructed from serial sections, related to Figure 6C. ZsGreen⁺ cells in green, tdTomato⁺ cells in red and DAPI in blue

Video 6. Panoptic multicolor imaging of ZsGreen⁺ cells (green) & tdTomato⁺ cells (red) in the pulp and PDL area mandible M1 of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (tracing 11 days), the whole-tissue imaging was reconstructed from serial sections, related to Figure 7C. ZsGreen⁺ cells in green, tdTomato⁺ cells in red and DAPI in blue **Video 7**. Panoptic multicolor imaging of PDGFR- a^+ cells (green) & NFATc1⁺ cells (red) in cranium of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse), the whole-tissue imaging was achieved through TC procedure.

Video 8. Panoptic multicolor imaging of PDGFR- α^+ cells (green) & NFATc1⁺ cells (red) in cranial sagittal suture of $Pdgfr\alpha^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse), the whole-tissue imaging was achieved through TC procedure.

Video 9. Panoptic multicolor imaging of PDGFR- a^+ cells (green) & NFATc1⁺ cells (red) in cranial coronal suture of $Pdgfra^{CreER} \times Nfatc1^{DreER} \times LGRT$ mice (pulse), the whole-tissue imaging was achieved through TC procedure.

Video 10. Panoptic multicolor imaging of ZsGreen⁺ cells in the pulp and PDL area of maxilla M1 of $Pdgfr\alpha^{CreER} \times IR1$ mice, the whole-tissue imaging was reconstructed from serial sections, related to Figure 8. ZsGreen⁺ cells in green and DAPI in blue.

Video 11. Panoptic multicolor imaging of tdTomato⁺ cells in the pulp and PDL area of maxilla M1 of $NFATc1^{DreER} \times IR1$ mice, the whole-tissue imaging was reconstructed from serial sections, related to Figure 8. tdTomato⁺ cells in red and DAPI in blue.

Video 12. Panoptic multicolor imaging of ZsGreen⁺ cells & tdTomato⁺ cells in the pulp and PDL area of maxilla M1 of $Pdgfra^{CreER} \times NFATc1^{DreER} \times IR1$ mice, the wholetissue imaging was reconstructed from serial sections, related to Figure 8. ZsGreen⁺ cells in green, tdTomato⁺ cells in red and DAPI in blue.