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Supplementary Materials for

Regeneration of pulpo-dentinal–like complex by a group of unique multipotent CD24a$^+$ stem cells

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Supplemental Materials

Figure Legends

Fig. S1. DPC spheres could be passaged without losing their self-renewal capability

(A) Characterization of batch variations for DPC spheres. The diameter of DPC spheres were quantified from three independent culture batches. Batch 1: n=102. Batch 2: n=149. Batch 3: n=113. (B) DPC Spheres could be passaged for at least two times. (C) Representative images of DPC spheres in 3D culture (left) and sphere-derived cells from spheres left (right). Scale bar: 200μm.

Fig. S2. DPC spheres were not derived from cell aggregates

(A) Schematic diagram of sphere formation assay. Primary cells from dental papilla were separately labeled with mCherry and EGFP by lentiviral transduction. Cells of two colors were then mixed together before seeded for 3D culture. (B) Fluorescence from DPC spheres indicated that the spheres were not derived from cell aggregates. Scale bar: 200μm.

Fig. S3. A small percentage of DPCs P2 positively expressed pluripotency markers including Oct4, Sox2 and Nanog

DPCsP2 were immunostained with antibodies against Oct4, Sox2, Nanog and Ki67. DAPI was used for nuclear staining. Scale bar:200μm.

Fig. S4. DPC spheres exhibited enhanced mineralization capability *in vitro* than DPSCs

AlizarinRed S (ARS) staining indicated DPC sphere-derived cells retained enhanced osteogenic differentiation capability compared with dental pulp stem cells (DPSCs). DPSCs, DPC Sphere-derived cells and DPCs P2 were incubated in osteo-inductive medium (OIM) for 3 days and 7 days respectively before harvested for ARS staining. Scale bar: 200μm.

Fig. S5.Cells derived from DPC Spheres exhibited enhanced osteogenic differentiation in *vivo* upon HA/TCP induction.

Monolayer cells, sphere-derived cells and DPCs P2 were combined with Matrigel plus HA/TCP respectively and transplanted under renal capsule for 4 weeks before harvest. The HE and Masson staining showed that mineralized structure formed in sphere-derived cells and in DPCs P2 group and monolayer group formed collagen fibers without mineralization. Scale bar: 50 μm.

Fig. S6. Cells derived from DPC spheres could form regenerated dentin structure in *vivo*.

Cells from DPC spheres were transplanted into TDM and subcutaneously in nude mice. Dentin-like structures were formed by these cells in two independent experiments. Scale bar: 200μm(left) and 50 μm(right).

Fig.S7. Cells derived from DPC spheres could form pulp-dentin-like structures in *vivo*.

Immunofluorescence staining supplementary images of specific antibodies. Dentin-related markers: DMP1, DSPP. Blood vessel-related markers: VEGF, CD31. Neural tissue-related markers: GFAP, S100 and NF-200. Scale bar: 50 μm.

Fig. S8. Bambi and Lgr6 were not enriched in DPC spheres.

Cells from monolayer culture, DPC spheres and DPCs at P2 were immunostained with anti-Bambi and anti-Lgr6 antibodies before taking image. All the images were acquired under the same exposure condition. Scale bar: 200μm.

Fig. S9. Knockdown of Sp7 significantly decreased DPC sphere formation in Balb/c mice.

(A) Efficient knockdown of Dlx3, Rcor2 and Sp7 by lentiviral shRNAs in primary cells from dental papilla of balb/c mice. Samples were harvested at day3 post transduction and RT-qPCR was used to evaluate knockdown efficiency. Error bar represents data from four replicates. (B) Sphere formation was strongly suppressed in Sp7-knockdown group while Dlx3 and Rcor2 did not seem to affect sphere formation. Representative images for at least two shRNAs were shown. Scale bar: 200 μm.

TableS1. Primer sequences.

Gene name	Forward	Reverse
GAPDH	5’-TTGTCAAGCTCATTTCCTGGT-3’	5’-GGCCATGTAGGCCATGAG-3’
SOX2	5’-CATGAGAGCAAGTACTGGCAA-3’	5’-CCAACGATATCAACCTGCATGG -3’
OCT4	5’-TTAAGAACATGTGTAAGCTGCG-3’	5’-GCATATCTCCTGAAGGTTCTCA-3’
Nanog	5’-CATAACTTCGGGGAGGACTTTC-3’	5’-GCTTCCAAATTCACCTCCAAAT-3’
Ki67	5’-CACAGAGAACAAAGGTGTGAAG-3’	5’-GGAGACTGCAGAGCTATTTTTG-3’
CD24	5’-TACTGCAACCAAACATCTGTTG-3’	5’-TAGAAGAGAGAGAGAGAGAGCC-3’
Lgr6	5’-CACCTCTGGCTGGATGACAATGC-3’	5’-GATGTGGCGGATATGGTTGAGAGC-3’
Bambi	5’-GGCAACAGGTTACATGTGTAAG-3’	5’-CGATAATTGCACATGTCTTCGT-3’
SP7	5’-TCCCTGGATATGACTCATCCCT-3’	5’-CCAAGGAGTAGGTGTGTTGCC-3’
Dlx3	5’-CCGAGGTTCGCATGGTGAA-3’	5’-AAGGCCAGATACTGGGCTTTC-3’
Rcor2	5’-TGCTTCTGTGGCATAAACACG-3’	5’-GGCTGGGAATCACCTTGTCAG-3’

TableS2. shRNA cloning sequences.

Gene name	Sense sequence	Anti-sense sequence
shDlx3-1	5’-CCGGCCTCACACAAACACAGGTGAACTCGAGTTCACCTGTGTTTGTGTGAGGTTTTTG-3’	5’-AATTCAAAAACCTCACACAAACACAGGTGAACTCGAGTTCACCTGTGTTTGTGTGAGG-3’
shDlx3-2	5’-CCGGCGTTTCCAGAAAGCCCAGTATCTCGAGATACTGGGCTTTCTGGAAACGTTTTTG-3’	5’-AATTCAAAAACGTTTCCAGAAAGCCCAGTATCTCGAGATACTGGGCTTTCTGGAAACG-3’
shSp7-1	5’-CCGGGCCATCTTAAACGTGCTCTTTCTCGAGAAAGAGCACGTTTAAGATGGCTTTTTG-3’	5’-AATTCAAAAAGCCATCTTAAACGTGCTCTTTCTCGAGAAAGAGCACGTTTAAGATGGC-3’
shSp7-2	5’-CCGGCCTGGATATGACTCATCCCTACTCGAGTAGGGATGAGTCATATCCAGGTTTTTG-3’	5’-AATTCAAAAACCTGGATATGACTCATCCCTACTCGAGTAGGGATGAGTCATATCCAGG-3’
shRcor2-1	5’-CCGGACCCAAGTGAAGACCTTCTTTCTCGAGAAAGAAGGTCTTCACTTGGGTTTTTTG-3’	5’-AATTCAAAAAACCCAAGTGAAGACCTTCTTTCTCGAGAAAGAAGGTCTTCACTTGGGT-3’
shRcor2-2	5’-CCGGCCCTGAGGCTAATACCAAGTTCTCGAGAACTTGGTATTAGCCTCAGGGTTTTTG-3’	5’-AATTCAAAAACCCTGAGGCTAATACCAAGTTCTCGAGAACTTGGTATTAGCCTCAGGG-3’

Fig. S1.

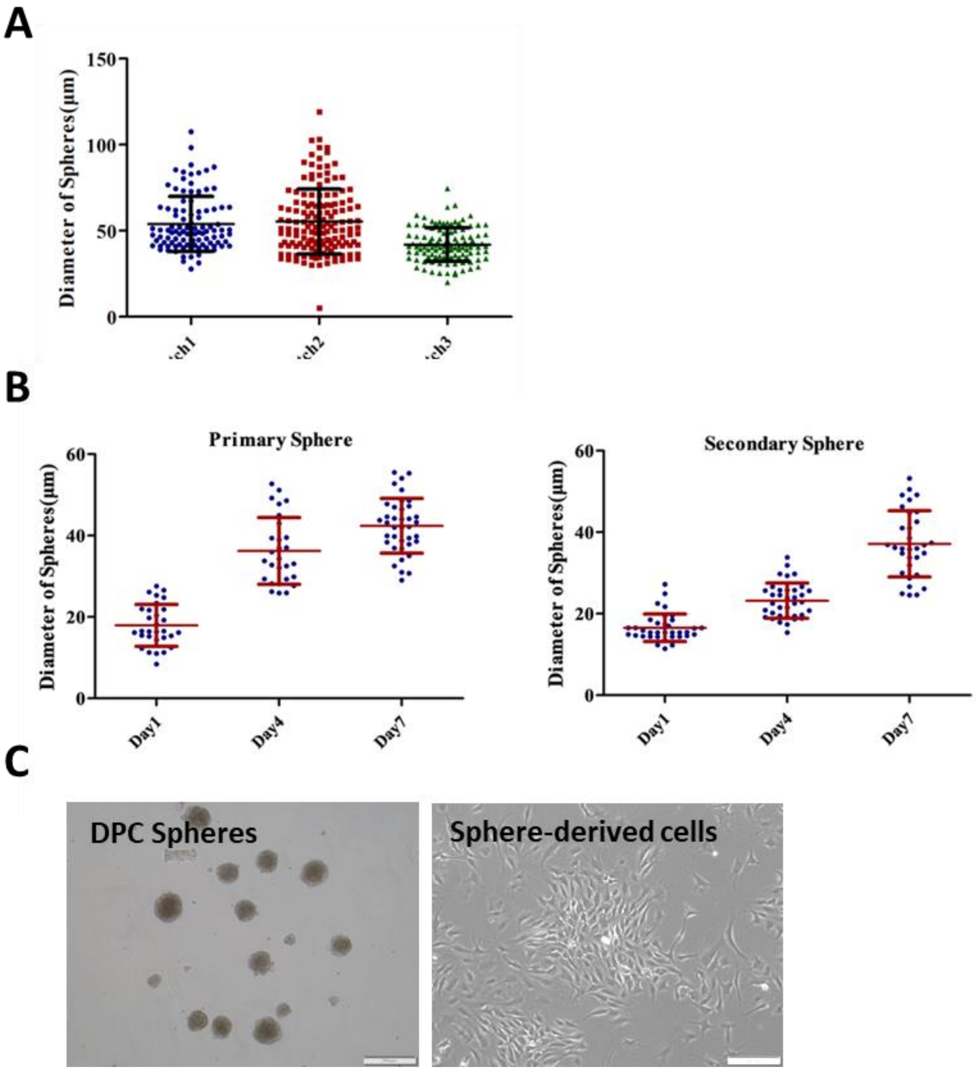

A
Diameter of Spheres(μm)
150
100
50
0
B
Primary Sphere
Secondary Sphere
Diameter of Spheres(μm)
60
40
20
0
Day1
Day4
Day7
C
DPC Spheres
Sphere-derived cells

Fig. S2.

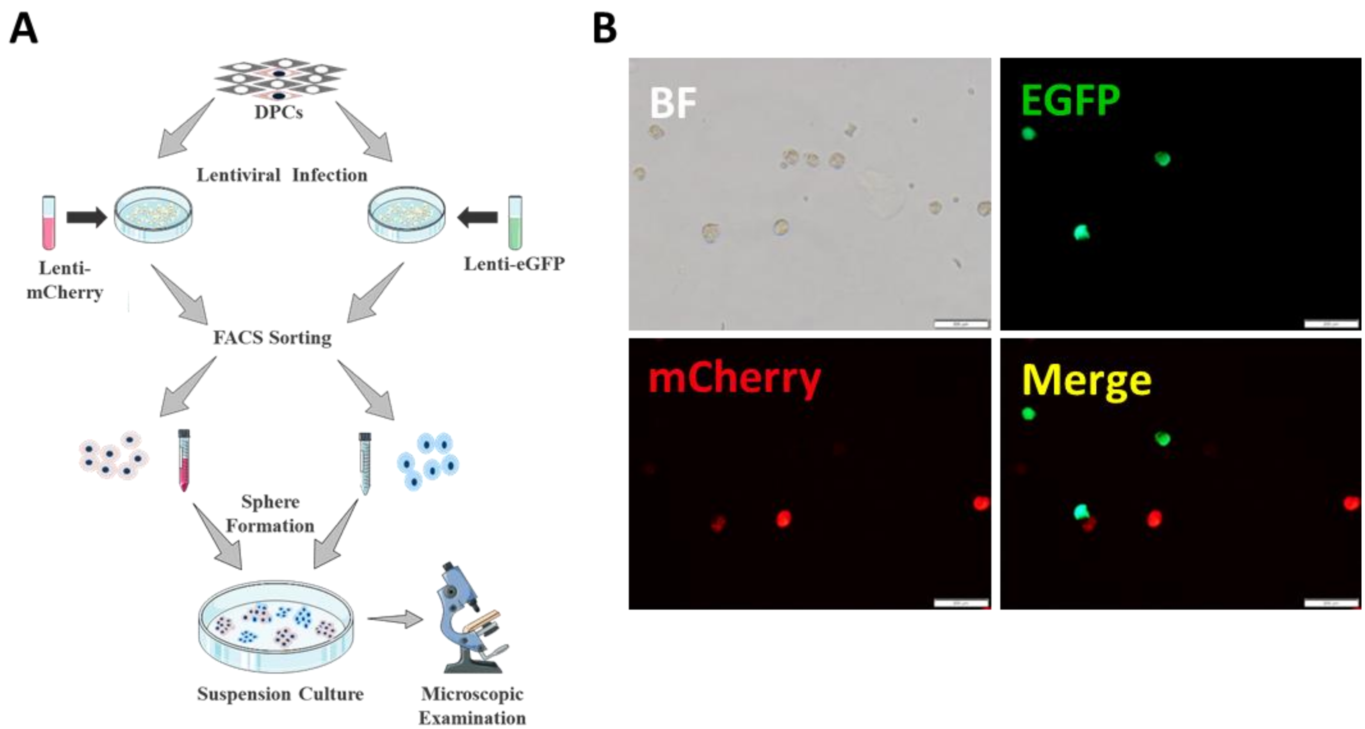

A
DPCs
Lentiviral Infection
Lenti-mCherry
Lenti-eGFP
FACS Sorting
Sphere Formation
Suspension Culture
Microscopic Examination
B
BF
EGFP
mCherry
Merge

Fig. S3.

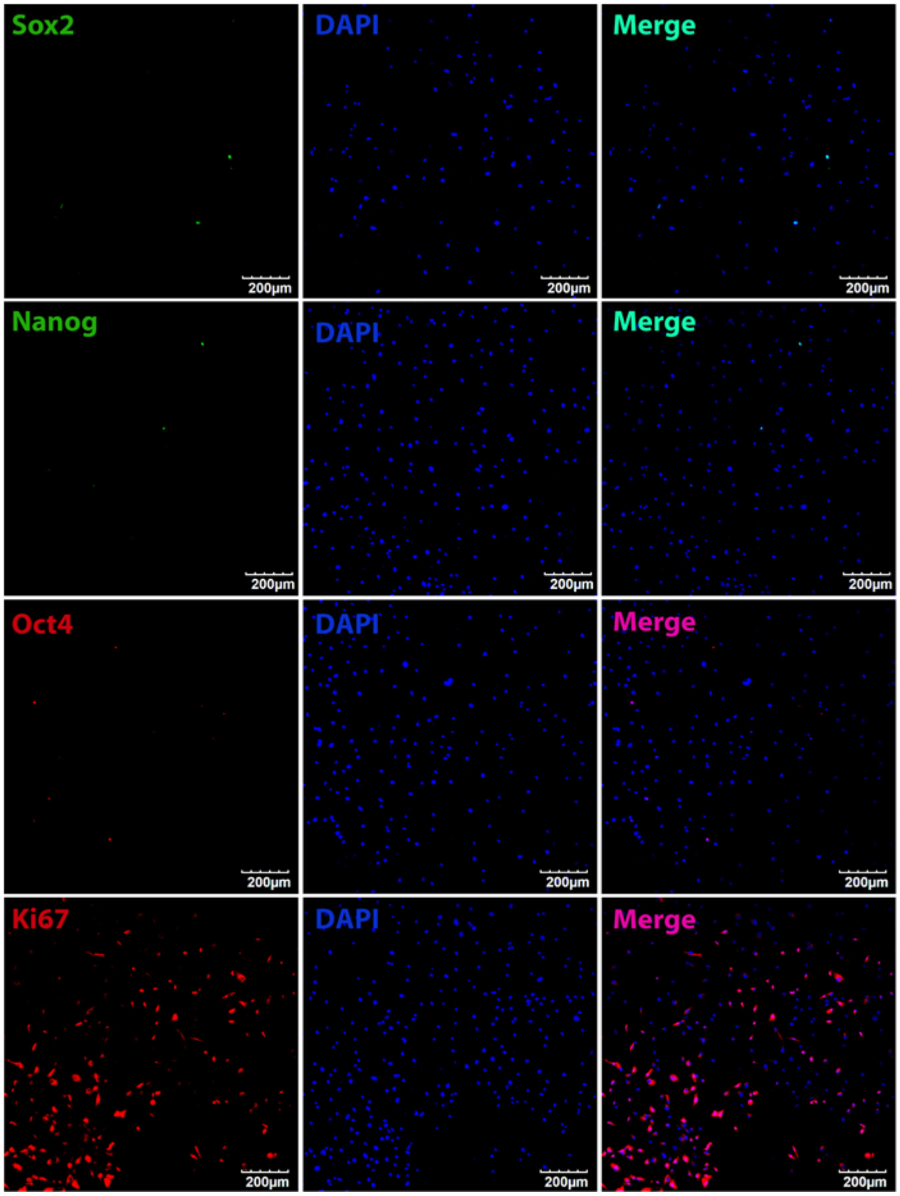

Sox2
DAPI
Merge
200μm
200μm
200μm
Nanog
DAPI
Merge
200μm
200μm
200μm
Oct4
DAPI
Merge
200μm
200μm
200μm
Ki67
DAPI
Merge
200μm
200μm
200μm

Fig. S4.

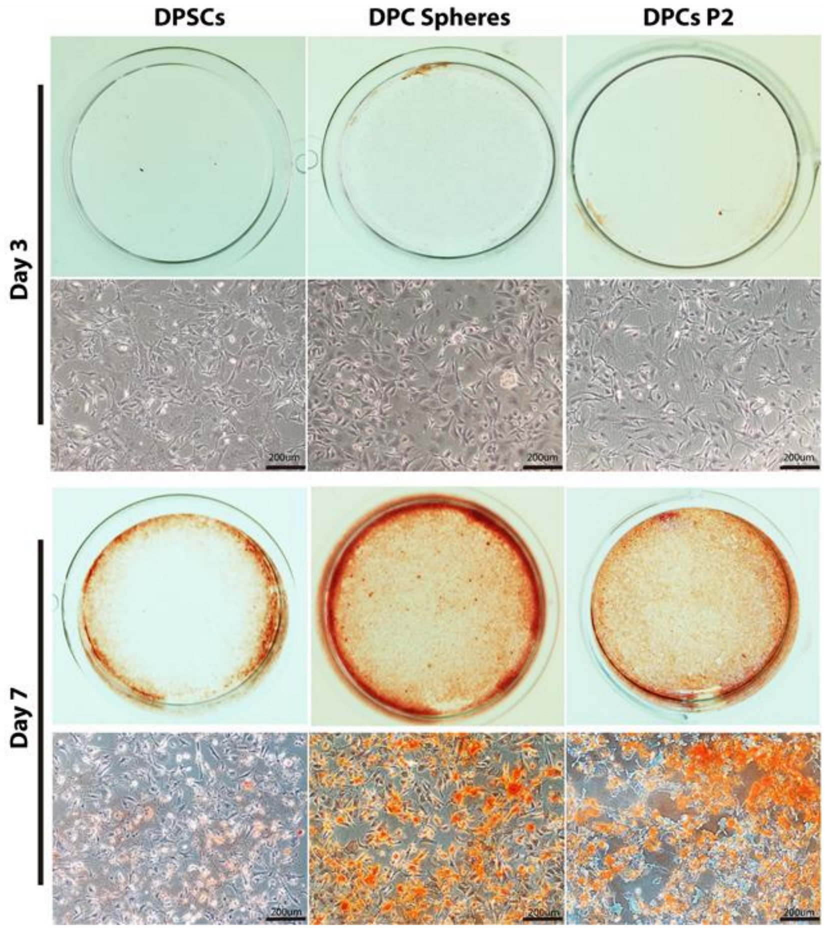
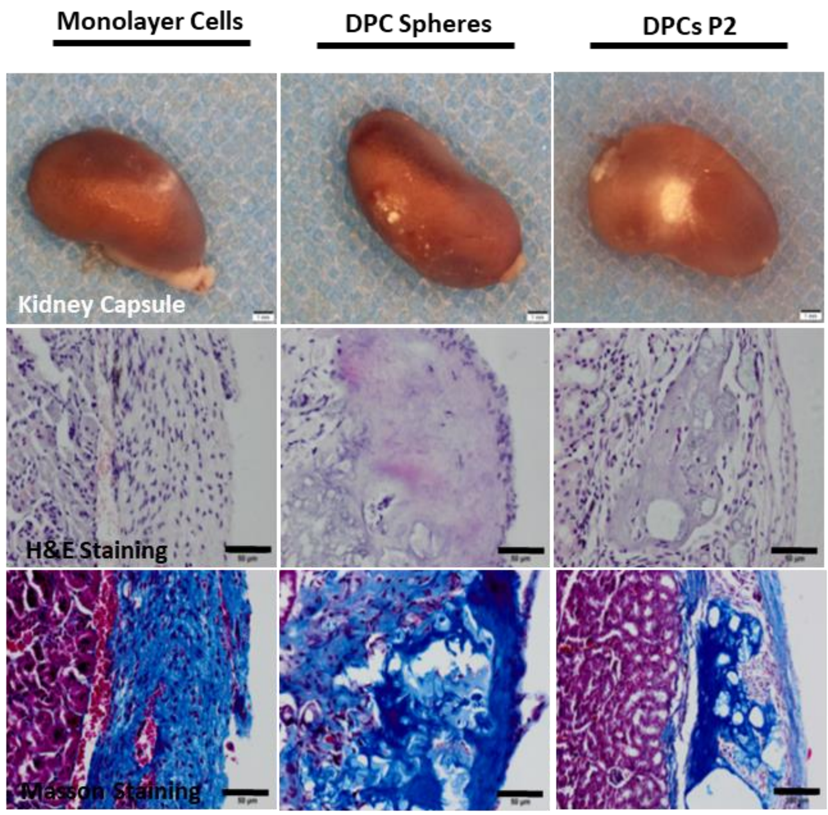


Fig. S5.



Monolayer Cells
DPC Spheres
DPCs P2
Kidney Capsule
H&E Staining
Masson Staining

Fig. S6.

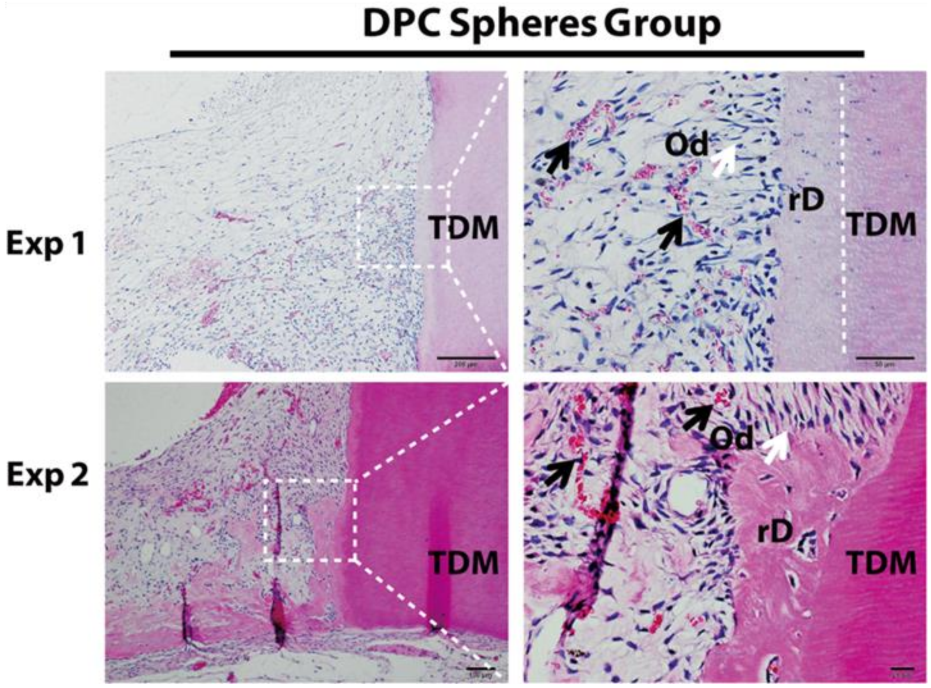


Fig. S7.

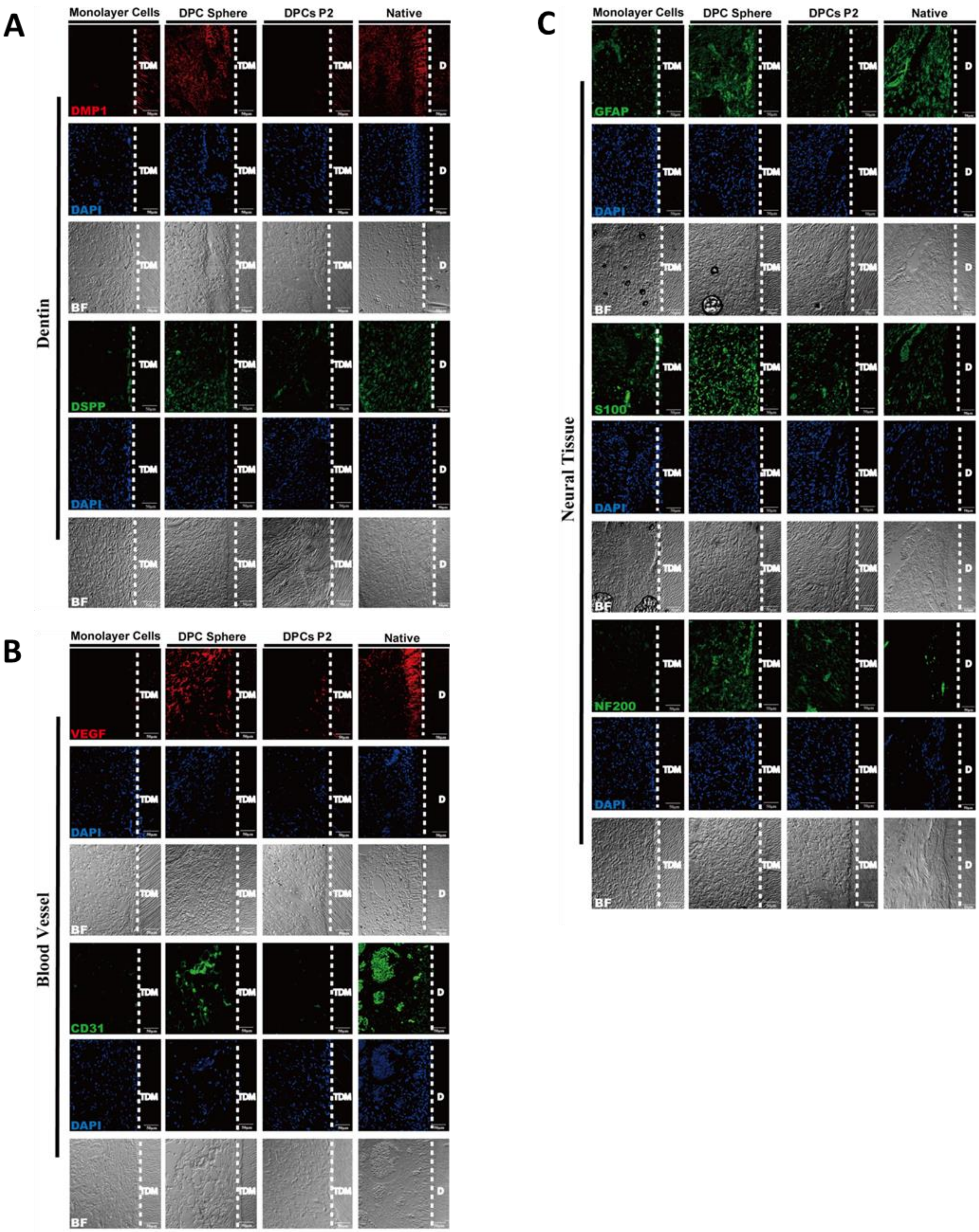

A
Monolayer Cells
DPC Sphere
DPCs P2
Native
TDM
D
DMP1
DAPI
BF
DSPP
Dentin
B
VEGF
CD31
Blood Vessel
C
GFAP
S100
NF200
Neural Tissue

Fig. S8.

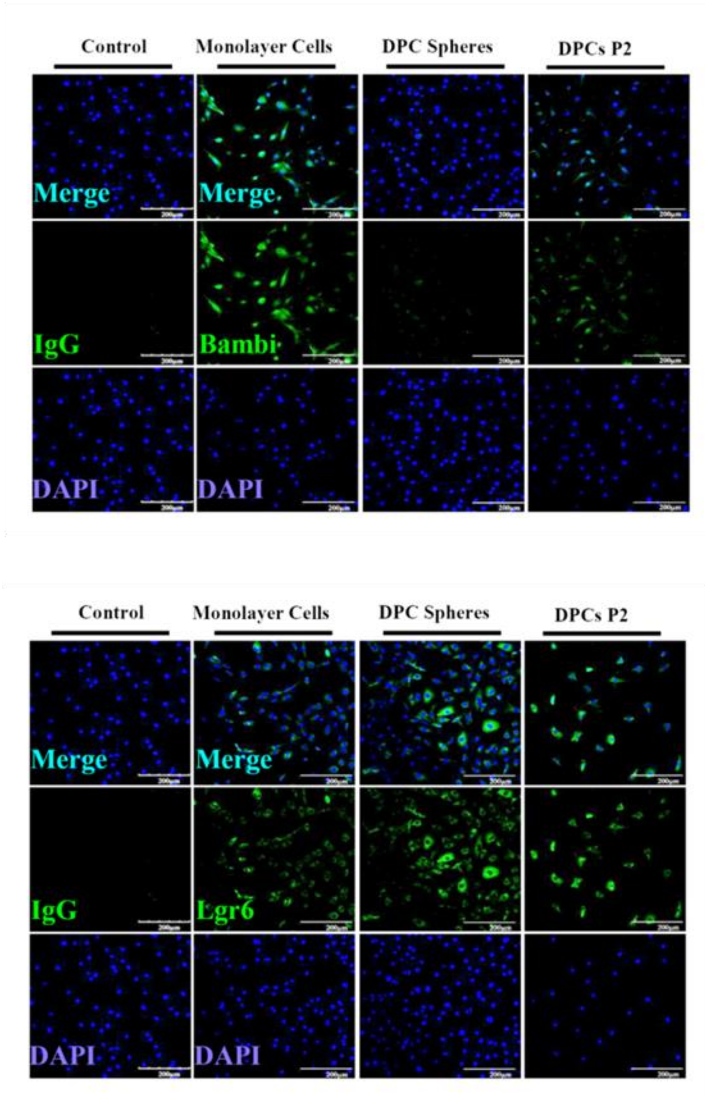
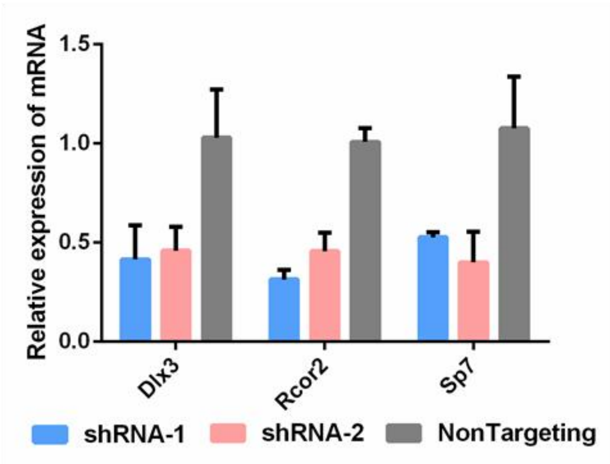
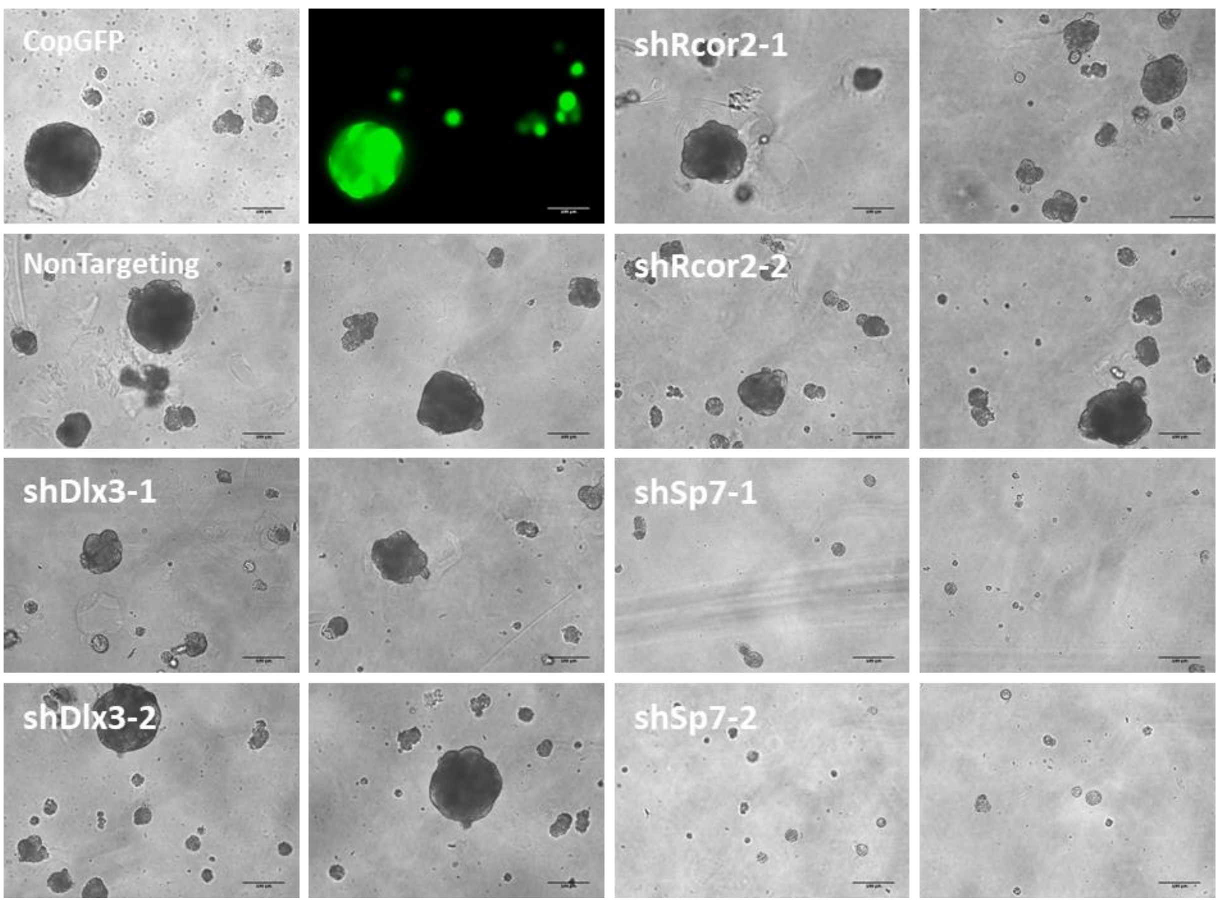

Control
Monolayer Cells
DPC Spheres
DPCs P2
Merge
Merge
IgG
Bambi
DAPI
DAPI
Control
Monolayer Cells
DPC Spheres
DPCs P2
Merge
Merge
IgG
Lgr6
DAPI
DAPI

Fig. S9.

A


Relative expression of mRNA
1.5
1.0
0.5
0.0
Dlx3
Rcor2
Sp7
shRNA-1
shRNA-2
NonTargeting

B


CopGFP
shRcor2-1
NonTargeting
shRcor2-2
shDlx3-1
shSp7-1
shDlx3-2
shSp7-2