Supplementary materials

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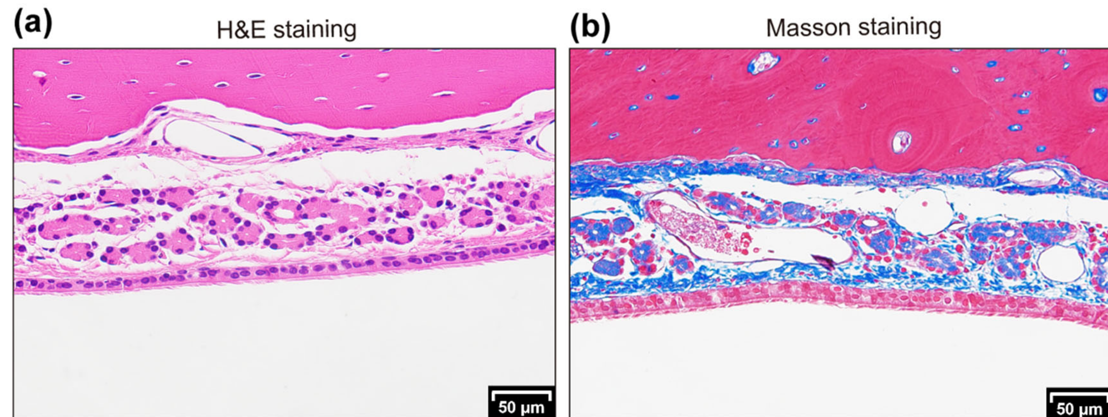

Fig. S1 The morphological observations of rabbit Schneiderian membrane. Representative histological images of H&E staining (a) and Masson trichrome staining (b) for the rabbit Schneiderian membrane. The rabbit Schneiderian membrane was composed of three layers: the upper cortex, lamina propria, and periosteal layer.

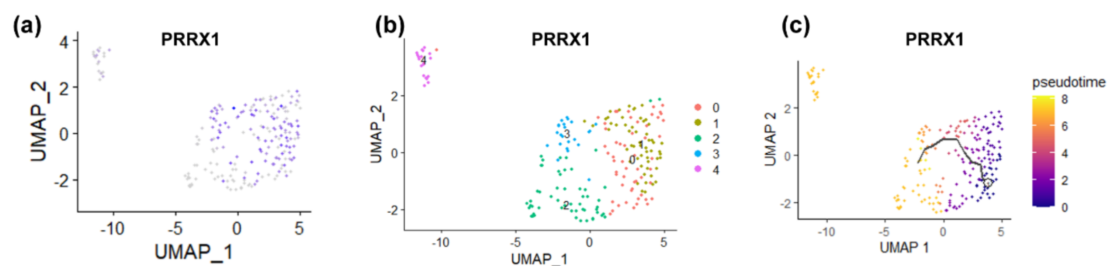

Fig. S2 Subclusters of PRRX1^{+} MSCs. (a) Expression of PRRX1 in mesenchymal stem cells (MSCs). (b) PRRX1^{+} MSCs were divided into 5 subclusters and each color represents each subcluster. (c) Pseudotime analysis of PRRX1^{+} MSCs. Colors represent corresponding developmental trajectories.

Method S1

Detailed parameters for single-cell RNA sequencing data analysis

Cell filtering criteria: Cells were filtered by (1) number of genes (<200), (2) UMI (<1000), (3) lgGenesPerUMI (<0.7), (4) percentage of mitochondrial RNA UMIs (>10%), and (5) percentage of hemoglobin RNA UMIs (>5%).

Normalization and variable gene selection: Library size normalization was processed using the NormalizeData function. The top 2000 highly variable genes were identified using FindVariableGenes (mean.function=FastExpMean, dispersion.function=FastLogVMR).

Dimensionality reduction and clustering: Principal-component analysis was run on the normalized gene-barcode matrix. Graph-based clustering was performed using the FindClusters function. Cells were visualized using UMAP with the RunUMAP function.

Differential expression: FindAllMarkers and FindMarkers functions were used with test.use=presto. Significance thresholds: $P<0.05$ and $|\log_2(\text{fold change})|>0.58$.

Method S2

Detailed trilineage induction medium compositions

Osteogenic medium: 10 mmol/L β-glycerophosphate, 50 μg/mL L-ascorbic acid, 10 nmol/L dexamethasone (all from Sigma).

Adipogenic medium: 10 mg/L insulin, 1×10^{-6} mol/L dexamethasone, 0.45 mmol/L isobutyl methylxanthine, 500 mmol/L indomethacin (all from Sigma).

Chondrogenic medium: 10 μg/L transforming growth factor-β1, 50 μg/mL vitamin C, 1×10^{-8} mol/L dexamethasone, 1 mmol/L pyruvate (all from Sigma).

All media were refreshed every 3 days for 21 days.

Method S3

Antibody dilutions

Primary antibodies: OPN (Thermo Fisher, MA5-17180), ADN (Thermo Fisher, MA1-054), COL-II (DSHB, II-II6B3), PRRX1 (LSBio, LS-B2380, 1:100).

Secondary antibodies: Fluorescence-conjugated secondary antibodies (1:200, volume ratio); donkey anti-goat Alexa Fluor 488 (Thermo Fisher, A21206, 1:200).

Method S4

Detailed surgical procedures

Sinus floor elevation: Sodium pentobarbital (30 mg/kg) was intravenously injected for general anesthesia. Local anesthesia was provided using 2% lidocaine with 1:100,000 epinephrine. A 50-mm mid-sagittal incision was made at the nasal dorsum, and a full-thickness flap was elevated. Under continuous refrigeration with 0.9% sterile saline, two bone windows were made bilaterally with a 4-mm diamond burr. The Schneiderian membrane was elevated using microsurgical instrumentation. Bilateral sinus sites were assigned randomly to Bio-Oss® or 5% GelMA hydrogel. The surgical field was covered by BioGide. The periosteum was closed with absorbable suture 4-0, and the skin with 3-0 silk. Rabbits received 0.2 mL/kg penicillin for 3 days postoperatively.

Cranial bone defect:

A 4-cm sagittal midline scalp incision was made. Four circular calvarial defects (8 mm diameter, 2 mm deep) were created in the parietal bones using a trephine burr with saline irrigation, preserving the dura mater. Defects were randomly allocated to four interventions. A BioGide membrane was placed over the defect site. Periosteum and skin were closed in layers using 4-0 Vicryl. Rabbits received penicillin (0.2 mg/kg) for 3 days postoperatively.