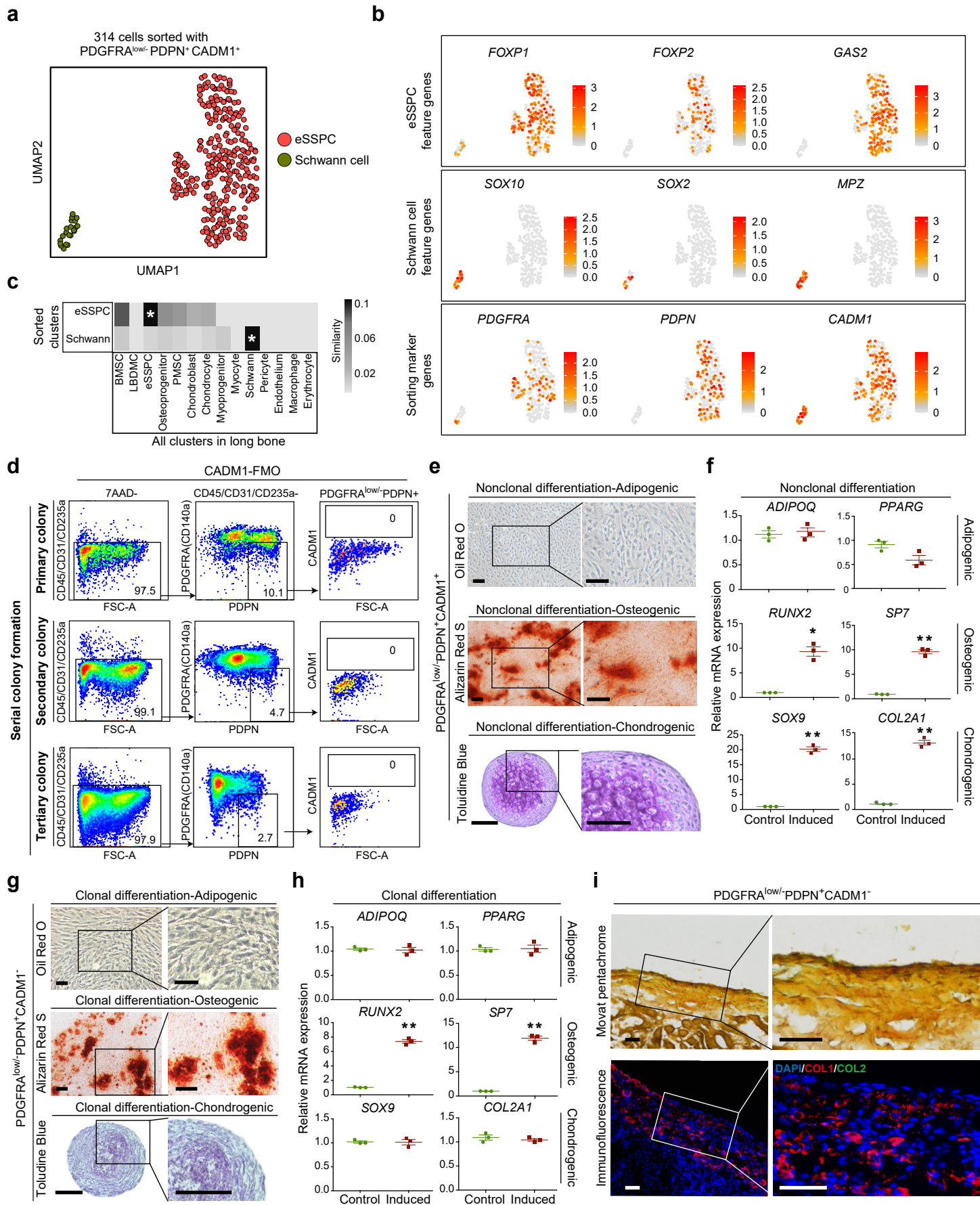


Figure S5



Supplementary Fig. 5. Validation of eSSPC phenotypic markers by scRNA-seq and further characterizations of PDGFRA^{low/-}PDPN⁺CADM1^{+/-} cells

a, Distribution of 314 PDGFRA^{low/-}PDPN⁺CADM1⁺ cells sorted from 8 WPC long bone. eSSPC and Schwann cell subsets were visualized by UMAP (n=1 embryo).

b, UMAP plots showing the expression of eSSPC feature genes (top), Schwann cell feature genes (middle) and sorting marker genes (bottom) in the cells of Fig.S5a.

c, Heatmap showing the Jaccard similarity of eSSPC and Schwann cell subsets in Fig.S5a and cell subsets of 8 WPC long bone datasets in Fig.S3a. Jaccard similarity was calculated using DEGs from different datasets. Asterisks indicated subsets with similarity > 0.1.

d, Fluorescence-minus-one (FMO) controls for eSSPC gating strategy in serial colony formation assay (Fig. 5a).

e and **f**, Representative oil red O (top), alizarin red S (middle) and toluidine blue (bottom) staining after adipogenic, osteogenic and chondrogenic differentiation of nonclonally expanded eSSPCs (PDGFRA^{low/-}PDPN⁺CADM1⁺) (**e**) with qPCR quantifications (**f**) (n=3 embryos). Magnified images of the boxed areas were shown on the right. Scale bars: 200 μ m.

g and **h**, Representative oil red O (top), alizarin red S (middle) and toluidine blue (bottom) staining after adipogenic, osteogenic and chondrogenic differentiation of clonally expanded PDGFRA^{low/-}PDPN⁺CADM1⁻ cells (**g**) with qPCR quantifications (**h**) (n=3 clones). Magnified images of the boxed areas were shown on the right. Scale bars: 200 μ m.

i, Renal subcapsular transplantation of PDGFRA^{low/-}PDPN⁺CADM1⁻ cells. Movat pentachrome staining (top, bone and fibrous tissue: yellow) and immunofluorescent staining images (bottom, DAPI: blue, collagen I (COL I): red, collagen II (COL II): green) were shown (n=4 grafts from one embryo). Magnified images of the boxed areas were shown on the right. Scale bars: 50 μ m.

The statistical significance of differences was determined using Wilcoxon signed rank test.

* $P < 0.05$; ** $P < 0.01$. Error bars indicated SEM.